Ethnobotanical studies on herbal remedies for musculoskeletal and neurological disorders in the Western Ghats region of Dakshina Kannada District, Karnataka, India.

Final synopsis

Submitted to

Mangalore University,

Mangalagangothri

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March 2024

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Registration number: MU/EXB.Ph.D/CR.79/A.B/2019-20/E-13

Registration date: 11th April 2018.

INTRODUCTION

From time immemorial, man has been depending on nature's plant diversity for all his basic needs. Man's preliminary interest in plants started from his need for food, shelter, protection and then his attention shifted to the remedies for various diseases and disorders. India has medicinal plants distributed in different geographical and environmental conditions. In India, the traditional healthcare system has a long history and is very deeply rooted among rural and tribal populations. India has the second largest tribal population in the world after Africa (Rai & Lalramnghinglova, 2011). Many tribal and ethnic communities in India still practice use of their traditional knowledge to treat a variety of diseases and ailments. Because these are considered to be safe, effective and inexpensive, indigenous remedies are gaining popularity among the people of both the urban and rural areas (Katewa *et al*., 2004).

India is one among the 12 mega biodiversity countries of the world due to its species richness and is inhabited by more than 550 tribal and several ethnic communities with a rich source of traditional knowledge of using plants available locally (Lingaraju *et al*., 2013). This knowledge naturally enriched through generations and passed on from one generation to another with or without any written documents. In most of the countries the knowledge remained without written records and often this precious knowledge has been lost due to lack of proper documentation. The researchers were interested in the cultural attributes of primitive societies and organized studies were initiated (Silambarasan & Ayyanar, 2015). Since then the proper documentation and preservation of traditional knowledge especially on the medicinal uses of the plants has started and a new branch of biology - "Ethnobotany" was evolved. In fact, medicine and botany always had strong and close connections, as most of the modern medicines come from plant sources (Savinaya *et al*., 2016).

The term ‘ethnobotany’ was first coined by Harshberger (1896) in his publication ‘The purposes of ethnobotany’. The concept of ethnobotany has a wide range of disciplines such as ethnomedicine, ethnopharmacology, ethnoalgology, ethnomycology, ethnobryology, ethnopteridology and ethnolichenology (Jain, 1987). The term ethnomedicobotany is represented by various synonyms such as ethnomedicine, traditional medicine, indigenous medicine, folk medicine and herbal medicine (Kumar & Ashaq, 2021).

The World Health Organization (WHO) defines traditional medicine as “The sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health, as well as in the prevention, diagnosis, improvement or treatment of physical and mental illnesses” (Che *et al*., 2017). Today, ethnomedicobotany plays an important role to document traditional knowledge of tribes and ethnic communities that has been practiced as a family heirloom and also to validate the medicinal plants scientifically to reach the demand of drugs of natural origin (Taylor *et al*., 2001).

Disorders of the musculoskeletal system are the single largest group of illness in the developing and developed countries. The WHO reported that Musculoskeletal disorders (MSDs), such as osteoarthritis, arthritis, back pain and neck pain, and bone fractures are the second most common cause of disability in the world (Kantasrila *et al*., 2020). MSDs include a wide range of inflammatory and degenerative conditions affecting the muscles, tendons, ligaments, joints, peripheral nerves, and supporting blood vessels. These include clinical syndromes such as tendon inflammations and related conditions such as tenosynovitis, epicondylitis, bursitis, nerve compression disorders, and osteoarthritis, as well as less well standardized conditions such as myalgia, low back pain and other regional pain syndromes not attributable to known pathology.

MSDs are widespread in many countries, with substantial costs and impact on quality of life (Jahan *et al*., 2015). The conditions in these disorders are typically characterized by pain and limitations in mobility, dexterity, and functional ability, reducing working ability and participation in social activities with associated impacts on mental wellbeing, and at broader level impacts on the prosperity of communities. The most common and disabling conditions here are osteoarthritis, back and neck pain, fractures associated with bone fragility, injuries, and systemic inflammatory conditions such as rheumatoid arthritis (Malik *et al*., 2021).

Neurological conditions are associated with the nervous system, particularly the brain and the spinal cord, which plays an important role in performing vital functions. Various factors affect the structure or function of brain (encephalopathy) or spinal cord (myelopathy) causing neurological or psychiatric or neurodegenerative complications. Neuroprotection denotes strategies to defend the nervous system against a number of factors such as structural defects, infections, neuronal injury, autoimmune disorders, neurodegeneration and tumors, which may lead to nervous system disorders (Upadhyay, 2014). A new report from the WHO have shown that neurological disorders affect up to one billion people worldwide, among them 6.8 million people die every year. In addition, the prevalence of CNS disorders is around two times higher in developing countries than in the developed world (Uddin & Zidorn, 2020). Inadequate and inaccessible health facilities in the rural and remote areas have forced the various tribes and non-tribal communities to use herbal therapeutics against different ailments (Amoateng *et al*., 2018).

The incidences of musculoskeletal and neurological problems are common among the people across the globe. People particularly in the developing countries, manage and overcome these problems by traditional herbal treatments available locally. The Western Ghats region of Dakshina Kannada district, Karnataka harbors a rich diversity of medicinal plants. Apart from

the modern approach, traditional approaches to treat these disorders are of common occurrence in remote rural areas. These are age old practices evolved and orally transferred to generations over a period of time. Hence, this survey has been undertaken to document the ethnomedicinal practices to treat such disorders.

Objectives

The proposed work had the following objectives

- Documentation of ethno-medico-botanical knowledge utilized for the treatment of musculoskeletal and neurological disorders in the Western Ghats region of Dakshina Kannada district, Karnataka, India.
- Identification of documented plant species and preparation of voucher specimens.
- Phytochemical screening and *in-vitro* pharmacological studies on ethno-medicinal plants namely, *Litsea wightiana* and *Naravelia zeylanica*.

MATERIALS AND METHODS

Ethno-medico-botanical data collection

Information pertaining to traditional treatment methods for neurological and musculoskeletal disorders in Western Ghats region of Dakshina Kannada district was documented from local healers in different seasons between 2018 and 2023. Medical terms for tribal terminology of different symptoms and ailments were identified by consulting allopathic medical practitioners. Finally all the diseases were categorized and tabulated following the standard method given by Cook (1995) with some necessary modifications based on the findings. The informants were selected from remote forest and village areas on the basis of their reputation and ability to demonstrate traditional medicinal knowledge. Before the interviews, verbal, prior, informed

consent of each of the informants was obtained. Since the knowledge is a natural wealth of the local people, they were assured that the data would be used only for academic purposes. After explaining the purpose of our study, we recorded information on medicinal plants and their use, plant parts used, diseases treated, the preparation and administration methods, and the conservation status. Ethnobotanical data were obtained by means of semi-structured interviews and questionnaires based on standard methods (Martin, 2010**).** Interviews were conducted in local Tulu and Kannada language. Plants collected with the herbal healers during the guided field walk were authentically identified with the help of published floras such as Flora of South Kanara (Bhat, 2014), Flora of Presidency of Madras (Gamble, 1934) and Flora of Karnataka (Saldanha, 1984). The valid names of the plants were updated visiting World Flora Online (https://www.worldfloraonline.org), Plants of the World Online (https://powo.science.kew.org) and the international plant name index (www.ipni.org). All medicinal plants recorded were photographed in the field and the voucher specimens were made subsequently and are deposited in the Herbarium, Department of Applied Botany, Mangalore University, Mangalore.

Data processing and quantitative analysis

The collected data was analyzed systematically in the MS excel sheet. The information such as botanical name, local name, family name, part used and ethnomedicinal uses were attributed to each species. Identified plant species were checked for their present conservation status in the web database of International Union for Conservation of Nature (IUCN)–Red List of Threatened Species (https://www.iucnredlist.org/). Further, the various formulations used in the treatment of neurological and musculoskeletal disorders are also provided. A literature search on the mentioned plants was undertaken using Google Scholar, Scopus, and Pub Med for earlier ethnomedicinal reports and various pharmacological properties such as anti-inflammatory,

wound healing, antioxidant, anticonvulsant, antispasmodic, analgesics and antiepileptic activities.

Quantitative analysis of data have been carried out using standard ethnobotanical tools such as use value (Albuquerque *et al*., 2006), relative frequency of citation (Tardio & Pardo-de Santayana, 2008), Informant agreement ratio (Trotter & Logan, 2019), Informant Consensus Factor (Heinrich *et al*., 1998), Species therapeutic potential (Heinrich, 1998) and fidelity level (Friedman *et al*., 1986).

Phytochemical screening and *in-vitro* pharmacological analysis of selected ethnomedicinal plants

Among, 239 ethnomedicinal plants, *Litsea wightiana* (LW) and *Naravelia zeylanica* (NZ) were selected for phytochemical screening and *in-vitro* pharmacological studies.

In Indian system of medicine ayurveda, the plant *Naravelia zeylanica* has been used in the treatment of *pitta*, helminthiasis, dermatopathy, leprosy, rheumatalgia, odontalgia, colic inflammation, wounds and ulcers. From the ethnomedical reviews, it has been observed that the aerial parts of *Naravelia zeylanica* traditionally used in the treatment of *vata*, *pitta* and skin diseases. The leaves when crushed give a pungent odour which is inhaled to cure cold and all type of headaches including migraine. While root and stem paste is applied externally for psoriasis, itches and skin allergy. The root and stem have a strong penetrating smell and is used to relive malarial fever and headache (Raja Naika *et al*., 2015). Quantitative ethnobotanical analysis of the present study reported that *Naravelia zeylanica* has showed 100 % fidelity level for treating migraine. It was cited by maximum number of practitioners and exhibited high

informant agreement ratio. It is considered as an important species used for the treatment of migraine based on STP parameter.

Similarly, *Litsea wightiana* is a moderate sized tree in the family Lauraceae. It is endemic to Western Ghats of India, in the states of Tamil Nadu, Maharashtra, Kerala, and Karnataka (Bhat, 2014). *Litsea wightiana* has exhibited 100 % fidelity level for treating bone fracture with high relative frequency citation value and high informant agreement ratio. Also, this species is considered as therapeutically potential species for treating bone fracture based on STP parameter.

Preparation of extract

Litsea wightiana methanolic extract (LWm) was prepared by extracting dried bark powder of LW in 250 ml of methanol using the soxhlet apparatus. *Litsea wightiana* aqueous extract (LWw) of dried bark powder of LW was prepared using the water bath. The extract was dried under vacuum at 45 ºC and stored at 4 ºC for further analysis. Similarly, *Naravelia zeylanica* methanolic extract (NZm) was prepared by extracting dried leaf powder of NZ in 250 ml of methanol using the soxhlet apparatus. *Naravelia zeylanica* aqueous extract (NZw) of dried leaf powder of NZ was prepared using the water bath and then the extract was dried under vacuum at 45 ºC and stored at 4 ºC for further analysis. The yield of each extract was calculated and expressed in percentage.

The qualitative analysis was carried out following method given by Harborne (1998). The quantitative estimation of the polyphenols was done using the folin–reagent spectrometric method (Khanal *et al*., 2015). Using the aluminium chloride spectrometric technique, the total flavonoid content was calculated (Amoussa *et al*., 2015). The total tannins are estimated by

Folin-Denis method (Sadasivam, 1996). Antioxidant activity of the extract was studied by DPPH free radical scavenging assay (Sethi *et al*., 2020) and reducing power assay (Sethi *et al*., 2020). Nitric oxide radical scavenging activity was determined following to the method given by Garrat (1964). The *in-vitro* anti-inflammatory activity of the plant extracts was determined following the albumin denaturation inhibition method (Sakat *et al*. 2010; Mizushima & Kobayashi, 1968). The phytoconstituents present in the extract was analyzed by GC-MS technique.

HR-LCMS analysis was performed to identify natural compounds present in LW bark aqueous extracts. Estimation of polysaccharides from the mucilage obtained from bark powder of LW was carried out following the method given by Yang *et al*. (2015).

RESULTS

Ethnobotanical studies

Western Ghats region of Dakshina Kannada district have diverse vegetation with different tribal and ethnic communities. People of these communities have a rich knowledge on using medicinal plants found around them for treating various ailments. The study revealed the use of 239 plant species belonging to 74 families in herbal medicines to treat various neurological and musculoskeletal disorders by 162 informants belonging to different ethnic communities in the district. Among 239 plant species recorded, 237 are angiosperms, while one species of gymnosperm and one belong to pteridophyte. Most of these plants used for the treatment are collected from the wild (176; 73.64%) and the rest of the species were cultivated (63; 26.36%). Among the families that contributed more medicinal species were Fabaceae (26 species), Apocyanaceae (11 species), Rubiaceae (10 species), Phyllanthaceae and Malvaceae (9 species

each), Rutaceae, Lamiaceae, Euphorbiaceae and Acanthaceae (8 species each), Moraceae, Asteraceae, Apiaceae, Myrtaceae and Solanaceae (6 species each), Poaceae and Lauraceae (5 species each), Combretaceae, Menispermaceae, Zingiberaceae and Oleaceae (4 species each), Anacardiaceae, Arecaceae, Calophyllaceae, Clusiaceae, Orchidaceae, Vitaceae, Aristolochiaceae, Piperaceae and Rhamnaceae (3 species each).

Habit-wise analysis of the plants indicates that trees are mostly used in the preparation of medicines (89) followed by herbs (66), climbers (43) and shrubs (41). Among the plant parts used by the practitioners, in most of the cases leaves are frequently utilized (39.47%), followed by bark (17.11%), root (13.42%), seed (8.68%), fruit (5.78%), stem (5.26%), whole plant (5.00%), flower (1.32%), rhizome and shoot tip (0.79% each), bulb, endosperm, latex and heart wood (0.53% each) and prop root (0.26%).

In the present study a total of 541 formulations have been recorded. Treatments are done either with single plant formulation (monoherbal) or combinations of several plants (polyherbal). Among the formulations, 389 are polyherbal formulations and remaining 152 are monoherbal. The different kinds of formulations are oil (36.97%), paste (29.39%), decoction (17.74%), juice (9.06%), powder (3.33%), crude form (0.93%), tablet and herbal gruel (0.74% each), ash (0.55%), lehya (0.37% each) and latex (0.18%). Practitioners used different ingredients such as rice washed water, rice water, butter milk, lemon juice, egg, jaggery, sugar, honey, oil, ghee, cow's milk and red ants as base material to prepare drug formulations. From the survey it was clear that most of the traditional practitioners recommended dermal applications (74.12%), followed by oral (18.48%), nasal (7.02%), nail gap and ear administrations (0.19% each).

The various parameters such as use value, informant agreement ratio and relative frequency of citation values of each plant species have been discussed. Similarly, the Informant consensus factor indicated the relative importance of species and greater homogeneity in sharing knowledge of herbal treatment in this region. Therapeutically potential plant species for each category of neurological and musculoskeletal disorders have been categorized. Claims are recorded for simple muscle pain to severe paralysis, indicating their strong knowledge in curing a range of diseases using different herbs. The present study quantifies the indigenous knowledge of different communities in the district and utilization of many important plants in the effective treatment of different types of neurological and musculoskeletal disorders.

Phytochemical screening and *in-vitro* pharmacological studies

Among 239 plant species, *Naravelia zeylanica* (NZ) and *Litsea wightiana* (LW) were selected for phytochemical screening because these species are the top prioritized plants by traditional practitioners for the treatment of migraine and bone fracture respectively. Preliminary phytochemical studies of leaf methanol and aqueous extract of NZ showed the presence of phenolics, alkaloids, flavonoids, tannins, saponins, coumarins, terpenoids, quinones and steroids while, Bark methanol extract and aqueous extract of LW showed the presence of phenolics, alkaloids, flavonoids, tannins, quinones, terpenoids and steroids.

Secondary metabolites of plants in varied concentrations are attributed to their antioxidant potential and hence are free radical scavengers. DPPH antioxidant activity and Ferric reducing assay in plant extracts was carried out. Results of the DPPH radical scavenging activity revealed that the methanol extract of LW has shown good antioxidant activity with IC_{50} value of 62.88 ± 0.15 µg/ml where as LWw extract showed IC_{50} value of 96.71 ± 4.8 µg/ml. The antioxidant

activity of NZ methanol and water extracts showed IC_{50} value of 708.58 ± 2.07 µg/ml and 595.55 ± 2.41 µg/ml respectively which suggests that leaves of NZ have shown a considerable amount of antioxidant activity.

LWm and LWw extracts showed reducing power absorbance of 1.15 nm and 1.06 nm at 0.5 mg/ml respectively. NZm and NZw have shown reducing power absorbance of 0.176 nm and 0.219 nm at 0.5 mg/ml respectively.

Nitric oxide scavenging activity revealed that LWw extract have exhibited better free radical scavenging activity with IC_{50} value of 153.627 ± 7.855 µg/ml whereas LWm showed IC_{50} value of 219.029 ± 3.676µg/ml. NZw showed IC_{50} value of 226.732 ± 12.21 µg/ml whereas NZm have exhibited an IC_{50} value of 310.214 ± 6 µg/ml.

Evaluation of the *in-vitro* anti-inflammatory activity by albumin denaturation inhibition method revealed that LWm, LWw, NZm and NZw extracts have shown significant anti-inflammatory activity compared to standard drug Ibuprofen. The LWm extract exhibited maximum inhibition of 74.61% at a concentration of 100 µg/ml and LWw extract showed the inhibition of 51.53% at a concentration of 100 µg/ml. Similarly, NZm extract showed maximum inhibition of 54.32% at a concentration of 100 µg/ml and NZw extract have exhibited maximum inhibition of 51.77% at 100 µg/ml. Ibuprofen, a standard anti-inflammatory drug, showed the maximum inhibition, 40.80% at a concentration of 100 µg/ml.

GC-MS studies on NZ leaf methanolic extract revealed the presence of natural compounds such as Neophytadiene; n-Hexadecanoic acid; Phytol; cis,cis,cis-7,10,13-Hexadecatrienal; Octadecanoic acid; Stigmasterol; γ-Sitosterol; Phthalic acid; butyl tetradecyl ester and Stigmast-7-en-3-ol, (3.beta.,5.alpha.,24S)- with potent antioxidant and anti-inflammatory activities.

GC-MS analysis of bark methanolic extract of LW showed the presence of p-Cresol, Octadecanoic acid and 13-Docosenamide, (Z)- with wound healing properties. Compounds such as Stigmastanol, γ-Sitosterol, Campesterol, Hordenine, Octadecanoic acid, n-Hexadecanoic acid, Palmitoleic acid and Pentadecanoic acid have shown potent anti-inflammatory properties.

GC-MS analysis of bark essential oil of LW showed the presence of 30 bioactive compounds such as Hexane, 2-nitro-; 2,3,5-Trimethylhexane; α-Cubebene; Copaene; Caryophyllene; Aromandendrene; γ-Muurolene; Cubebol; β-Cadinene; 7 epi-a-Selinene; Cubenene; Spatulenol; Caryophyllene oxide; Humulene epoxide-2; 1,10-Di-epi-Cubenol; tau-Cadinol; 2-Hydroxycyclopentadecanone; n-Hexadecanoic acid; Hexadecanoic acid, ethyl ester; 14-Methyl-8-hexadecyn-1-ol; Linoleic acid ethyl ester; Ethyl Oleate; Erucylamide; Vitamin E; Campesterol; Stigmasterol and γ-Sitosterol.

HR-LCMS analysis of bark aqueous extract of LW revealed the presence of various compounds namely, Rehmannioside-B; Cauloside-A; Benzoylgomisin-H; Kukoamine-B; Gomisin-G; Sophoradin; Ophiopogonanone-D; Piperlactam-S; Kushenol-C; Makisterone-A; Schisanhenol; (8)-Gingerol; alpha-Mangostin; Atractyloside-I; Futoquinol; Magnolin; Shikonin; Capitasterone; Lucenin-2 and Cnidimol-D. These compounds are responsible for antioxidant, anti-inflammatory, anti-arthritic, wound healing and analgesic properties of LW.

The bark powder of the plant LW has high mucilage content (28% w/w of dried bark), hence this plant can be taken as a good natural source of mucilage. Total polysaccharides content reported in LW bark is 488 mg/g of the mucilage.

HIGHLIGHTS

- Western Ghats region of Dakshina Kannada district, Karnataka state is the repository of ethnomedicinal knowledge due to large numbers of tribal and ethnic traditional practitioners and a wide variety of medicinal plants used to cure various types of human diseases and disorders.
- Ethnomedicinal data was collected by interviewing 162 traditional practitioners, of which 117 male practitioners and 45 female practitioners.
- Medicinal preparations have been documented for 7 major musculoskeletal disease categories such as arthritis, backache, bone fracture, joint pain, muscle pain, spasm and sprains as well as 7 neurological disease categories such as depression, epilepsy, insomnia, migraine, paralysis, psychosis and vertigo.
- Present study indicated that among the 239 plants recorded, 20 species are endemic to peninsular India and Western Ghats while 17 plants are under different threat categories. These plants should be protected from being lost and prioritized for conservation.
- Preliminary phytochemical analysis of leaf methanol and aqueous extract of *Naravelia zeylanica* showed the presence of phenolics, alkaloids, flavonoids, tannins, saponins, coumarins, terpenoids, quinones and steroids.
- Bark methanol extract and aqueous extract of *Litsea wightiana* showed the presence of phenolics, alkaloids, flavonoids, tannins, quinones, terpenoids and steroids. Presence of high phenolics is attributed to their good antioxidant properties.
- Bark aqueous extract of *Litsea wightiana* has shown good amount of polysaccharides.

- GC-MS studies on methanolic extract of *Litsea wightiana* showed the presence of various phenolic compounds and steroids which plays a key role in wound healing and anti-inflammatory activities.
- GC-MS studies of bark essential oil of *Litsea wightiana* showed the presence of various phenolic compounds which plays a key role in wound healing, analgesics and anti-inflammatory activities.
- GC-MS studies on methanolic leaf extract of *Naravelia zeylanica* showed the presence of various natural compounds responsible for anti-inflammatory and antioxidant activities.
- HR-LCMS of aqueous extract of *Litsea wightiana* has been carried out; results indicated the presence of 30 bioactive compounds in ES positive mode and 20 compounds in ES negative mode with potent anti-inflammatory, analgesics and wound healing properties.
- In general, the outcome of the present study is that it provides a scientific evidence for the use of medicinal plants by the traditional practitioners of Western Ghats region of Dakshina Kannada district for the treatment of neurological and musculoskeletal disorders. This may also provide a way for further studies in the treatment of neurological and musculoskeletal disorders.

List of publications:

1. Yogeesha, A., & Krishnakumar, G. (2023). Ethnobotanical study of medicinal plants used in the treatment of neurological disorders in the Western Ghats region of Dakshina Kannada district, Karnataka, India. *Ethnobotany Research & Applications*, 26(62), 1–30.

2. Yogeesha, A., & Krishnakumar, G. (2022). Folklore claims of medicinal plants against sprain from the Western Ghats region of Dakshina Kannada district, Karnataka state, India. *Biomedicine*, 42(2), 388–394.

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5.Yogeesha, A., & Krishnakumar, G. (2024). Ethnobotanical investigation on herbal remedies for musculoskeletal disorders in Dakshina Kannada district, Karnataka, India. *Ethnobotany Research & Applications*, (Communicated, March 2024).

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1. Yogeesha, A., & Krishnakumar, G. (2021). Ethno-botanical studies on traditional medicinal plants used by *Malekudiya* tribes in Subramanya region of Dakshina Kannada district, Karnataka, India. International congress of Society for Ethnopharmacology, India (SFEC 2021), Pune, India during 27th to 29th, August 2021 organized by SFE-India Pune Chapter & Bharathi

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Place: Mangalagangothri (Yogeesha A)

Date: 16- 03- 2024

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